

**WHAT IS CLAIMED IS:**

1. A method of determining the presence and identity of a variation in a nucleotide sequence between a first polynucleotide and a second polynucleotide, comprising:

a) providing a sample of the first polynucleotide;

5 b) selecting a region of the first polynucleotide potentially containing the variation;

c) subjecting the selected region to a template producing amplification reaction to produce a first plurality of double stranded polynucleotide templates which include the selected region;

10 d) selecting a region of the first polynucleotide sequence lying within the templates for analysis;

e) producing a family of labeled, linear polynucleotide fragments from both strands of the templates simultaneously by a fragment producing reaction including,

i) a primer pair,

15 ii) dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP, and

iii) two non-Watson-Crick-pairing dideoxyterminators;

where the primer pair flank the selected region of the template strands;

where each of the primer pair is labeled;

20 where at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP is labeled;

where each of the two non-Watson-Crick-pairing dideoxyterminators is labeled;

where each of the labels on the primer pair and labels on the two non-Watson-Crick-pairing dideoxyterminators are all distinguishable from each other;

25 where each of the family of labeled, linear polynucleotide fragments from both strands of the templates are terminated by one of the two labeled non-Watson-Crick-pairing dideoxyterminators at the 3' end of the fragment; and

) where the labeled, linear polynucleotide fragments from both strands of the templates include at least one fragment terminating at each possible base, represented by either of the two non-Watson-Crick-pairing dideoxyterminators of that portion of the selected region of both template strands flanked by one of the labeled primer pair; and

5 f) determining the location and identity of the bases in the selected region of the first polynucleotide by detecting the labels present in the fragments.

2. The method of claim 1, additionally comprising comparing the location and identity of the bases determined with the location and identity of bases from a second polynucleotide, thereby identifying the presence and identity of a variation in a nucleotide  
10 sequence between the selected region of the first polynucleotide and a corresponding region of the second polynucleotide, after determining the location and identity of the bases in the selected region of the first polynucleotide.

3. The method of claim 1, where the selected region of the first polynucleotide comprises a plurality of discontinuous sequences on the first polynucleotide.

15 4. The method of claim 1, where the template producing amplification reaction comprises subjecting the selected region to PCR.

5. The method of claim 1, where the template producing amplification reaction comprises subjecting the selected region to RT-PCR.

20 6. The method of claim 1, where the first plurality of double stranded polynucleotide templates comprise double stranded nucleic acid strands of between about 50 and 50,000 nucleotides per strand.

7. The method of claim 1, further comprising purifying the templates to remove other amplification reaction components after subjecting the selected region to a template producing amplification reaction.

25 8. The method of claim 1, where the fragment producing amplification reaction comprises subjecting the selected region to PCR.

9. The method of claim 1, where the fragment producing amplification reaction comprises subjecting the selected region to RT-PCR.

10. The method of claim 1, where the selected region of the template strands is between about 100 and 1000 nucleotides per strand.

5 11. The method of claim 1, where the two non-Watson-Crick-pairing dideoxyterminators are 2'-3'-dideoxyterminators.

12. The method of claim 1, where one of the two non-Watson-Crick-pairing dideoxyterminators comprises a pyrimidine nucleotide and where another of the two non-Watson-Crick-pairing dideoxyterminators comprises a purine nucleotide.

10 13. The method of claim 1, where the two non-Watson-Crick-pairing dideoxyterminators are selected from the group consisting of ddATP:ddCTP, ddATP:ddGTP, ddCTP:ddTTP and ddGTP:ddTTP.

14. The method of claim 1, where the two non-Watson-Crick-pairing dideoxyterminators are selected from the group consisting of ddATP:ddCTP, ddATP:ddGTP, 15 ddCTP:ddUTP and ddGTP:ddUTP.

15. The method of claim 1, where at least one of the labels are selected from the group consisting of fluorescent labels, fluorescent energy transfer labels, luminescent labels, chemiluminescent labels, phosphorescent labels and photoluminescent labels.

16. The method of claim 1, where the portion of one of the dATP, dCTP, 20 dGTP and either dTTP or dUTP or both dTTP and dUTP that is labeled comprises between about 1% and about 10% of the total concentration of unlabeled dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP.

17. The method of claim 1, further comprising purifying the labeled reaction products from the fragment producing reaction before determining the location and identity of 25 the bases in the selected region of the first polynucleotide.

18. The method of claim 1, where the sequence of the corresponding region of the second polynucleotide is determined by:

g) providing a sample of the second polynucleotide;

h) selecting a region of the second polynucleotide which corresponds to the region of the first polynucleotide potentially containing the variation;

i) subjecting the corresponding region of the second polynucleotide to a template producing amplification reaction to produce a second plurality of double stranded polynucleotide templates which include the corresponding region;

j) producing a family of labeled, linear polynucleotide fragments from both strands of the template simultaneously by a fragment producing reaction including,

i) a primer pair,

ii) dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP, and

iii) two non-Watson-Crick-pairing dideoxyterminators;

where the primer pair in step j) flank the selected region of the template strands;

where each of the family of fragments produced in step j) are terminated by either of the two non-Watson-Crick-pairing dideoxyterminators of step j) at the 3' end of the fragment; and

where the family of fragments include at least one fragment terminating at each possible base, represented by either of the two non-Watson-Crick-pairing dideoxyterminators of step j) of that portion of the selected region of both template strands flanked by a primer of step j);

k) determining the location and identity of at least some of the bases in the corresponding region of the second polynucleotide.

19. The method of claim 18, where the sequence of the corresponding region of the second polynucleotide is determined simultaneously with determining the location and identity of the bases in the selected region of the first polynucleotide.

20. The method of claim 18, where producing the family of labeled, linear polynucleotide fragments in step e) and producing the family of labeled, linear polynucleotide fragments in step j) is performed in one reaction.

21. The method of claim 18, where each of the primer pair in step j) is labeled, and the labels on each of the primer pair in step j) are all distinguishable from each other.

22. The method of claim 18, where at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step j) is labeled.

23. The method of claim 18, where each of the two non-Watson-Crick-pairing dideoxyterminators in step j) is labeled, and where each of the labels on the two non-Watson-Crick-pairing dideoxyterminators in step j) are all distinguishable from each other.

24. The method of claim 18, where each of the primer pair in step j) is labeled, at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step j) is labeled, and where the labels on each of the primer pair in step j) and the labels on at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step j) are all distinguishable from each other.

25. The method of claim 18, where each of the primer pair in step j) is labeled, each of the two non-Watson-Crick-pairing dideoxyterminators in step j) is labeled, and where the labels on each of the primer pair in step j) and each of the labels on the two non-Watson-Crick-pairing dideoxyterminators in step j) are all distinguishable from each other.

26. The method of claim 18, where at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step j) is labeled, each of the two non-Watson-Crick-pairing dideoxyterminators in step j) is labeled, and where the labels on at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step j) and each of the labels on the two non-Watson-Crick-pairing dideoxyterminators in step j) are all distinguishable from each other.

27. The method of claim 18, where each of the primer pair in step j) is labeled, at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP

and dUTP in step j) is labeled, each of the two non-Watson-Crick-pairing dideoxyterminators in step j) is labeled, and where the labels on each of the primer pair in step j), the labels on at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step j), and each of the labels on the two non-Watson-Crick-pairing  
5 dideoxyterminators in step j) are all distinguishable from each other.

28. A method of determining the presence and identity of a variation in a nucleotide sequence between a first polynucleotide and a second polynucleotide, comprising:

a) providing a sample of the first polynucleotide;

10 b) selecting a region of the first polynucleotide potentially containing the variation;

c) subjecting the selected region to a template producing amplification reaction to produce a first plurality of double stranded polynucleotide templates which include the selected region;

15 d) selecting a region of the first polynucleotide sequence lying within the templates for analysis;

e) producing a family of labeled, linear polynucleotide fragments from both strands of the templates simultaneously by a fragment producing reaction including,

i) a primer pair,

20 ii) dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP, and

iii) two non-Watson-Crick-pairing dideoxyterminators;

where the primer pair flank the selected region of the template strands;

25 where each of the family of labeled, linear polynucleotide fragments from both strands of the templates are terminated by one of the two non-Watson-Crick-pairing dideoxyterminators at the 3' end of the fragment; and

where the first family of fragments include at least one fragment terminating at each possible base, represented by either the first terminator or the second terminator of that portion of the selected region of both template strands flanked by a primer; and

where the labeled, linear polynucleotide fragments from both strands of the templates include at least one fragment terminating at each possible base, represented by either of the two non-Watson-Crick-pairing dideoxyterminators of that portion of the selected region of both template strands flanked by one of the primer pair; and

f) determining the location and identity of the bases in the selected region.

29. The method of claim 28, additionally comprising comparing the location and identity of the bases determined with the location and identity of bases from a second polynucleotide, thereby identifying the presence and identity of a variation in a nucleotide sequence between the selected region of the first polynucleotide and a corresponding region of the second polynucleotide, after determining the location and identity of the bases in the selected region of the first polynucleotide.

30. The method of claim 28, where each of the primer pair is labeled and where the labels on each of the primer pair are all distinguishable from each other.

31. The method of claim 28, where at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP is labeled.

32. The method of claim 28, where each of the two non-Watson-Crick-pairing dideoxyterminators is labeled, and where each of the labels on the two non-Watson-Crick-pairing dideoxyterminators are all distinguishable from each other.

33. The method of claim 28, where each of the primer pair is labeled, at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP is labeled, and where the labels on each of the primer pair and the labels on at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP are all distinguishable from each other.

34. The method of claim 28, where each of the primer pair is labeled, each of the two non-Watson-Crick-pairing dideoxyterminators is labeled, and where the labels on each of the primer pair and each of the labels on the two non-Watson-Crick-pairing dideoxyterminators are all distinguishable from each other.

5           35. The method of claim 28, where at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP is labeled, each of the two non-Watson-Crick-pairing dideoxyterminators is labeled, and where the labels on at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP and each of the labels on the two non-Watson-Crick-pairing dideoxyterminators are all  
10 distinguishable from each other.

36. The method of claim 28, where each of the primer pair is labeled, at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP is labeled, each of the two non-Watson-Crick-pairing dideoxyterminators is labeled, and where the labels on each of the primer pair, the labels on at least a portion of one of the dATP,  
15 dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP , and each of the labels on the two non-Watson-Crick-pairing dideoxyterminators are all distinguishable from each other.

37. The method of claim 28, where the selected region of the first polynucleotide comprises a plurality of discontinuous sequences on the first polynucleotide.

38. The method of claim 28, where the template producing amplification  
20 reaction comprises subjecting the selected region to PCR.

39. The method of claim 28, where the template producing amplification reaction comprises subjecting the selected region to RT-PCR.

40. The method of claim 28, where the first plurality of double stranded polynucleotide templates comprise double stranded nucleic acid strands of between about 50  
25 and 50,000 nucleotides per strand.



41. The method of claim 28, further comprising purifying the templates to remove other amplification reaction components after subjecting the selected region to a template producing amplification reaction.

42. The method of claim 28, where the fragment producing amplification reaction comprises subjecting the selected region to PCR.

43. The method of claim 28, where the fragment producing amplification reaction comprises subjecting the selected region to RT-PCR.

44. The method of claim 28, where the selected region of the template strands is between about 100 and 1000 nucleotides per strand.

45. The method of claim 28, where the two non-Watson-Crick-pairing dideoxyterminators are 2'-3'-dideoxyterminators.

46. The method of claim 28, where one of the two non-Watson-Crick-pairing dideoxyterminators comprises a pyrimidine nucleotide and where another of the two non-Watson-Crick-pairing dideoxyterminators comprises a purine nucleotide.

47. The method of claim 28, where the two non-Watson-Crick-pairing dideoxyterminators are selected from the group consisting of ddATP:ddCTP, ddATP:ddGTP, ddCTP:ddTTP and ddGTP:ddTTP.

48. The method of claim 28, where the two non-Watson-Crick-pairing dideoxyterminators are selected from the group consisting of ddATP:ddCTP, ddATP:ddGTP, ddCTP:ddUTP and ddGTP:ddUTP.

49. The method of claim 28, where each of the primer pair is labeled, at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP is labeled, each of the two non-Watson-Crick-pairing dideoxyterminators is labeled, and where the labels are selected from the group consisting of fluorescent labels, fluorescent energy transfer labels, luminescent labels, chemiluminescent labels, phosphorescent labels and photoluminescent labels.

50. The method of claim 28, where at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP is labeled and where the portion of labeled dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP comprises between about 1% and about 10% of the total concentration of unlabeled nucleotide triphosphates.

51. The method of claim 28, further comprising purifying the labeled reaction products from the fragment producing reaction before determining the location and identity of the bases in the selected region of the first polynucleotide.

52. The method of claim 28, where one or more of the primer pair, the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP, and the two non-Watson-Crick-pairing dideoxyterminators is labeled, and where determining the location and identity of the bases in the selected region of the first polynucleotide is accomplished by detecting the label or labels.

53. The method of claim 28, where the sequence of the corresponding region of the second polynucleotide is determined by:

g) providing a sample of the second polynucleotide;

h) selecting a region of the second polynucleotide which corresponds to the region of the first polynucleotide potentially containing the variation;

i) subjecting the corresponding region of the second polynucleotide to a template producing amplification reaction to produce a second plurality of double stranded polynucleotide templates which include the corresponding region;

j) producing a second family of labeled, linear polynucleotide fragments from both strands of the template simultaneously by a fragment producing reaction including,

i) a primer pair,

ii) dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP, and

iii) two non-Watson-Crick-pairing dideoxyterminators;

where the primer pair in step j) flank the selected region of the template strands;  
where each of the family of fragments produced in step j) are terminated by  
either of the two non-Watson-Crick-pairing dideoxyterminators of step j) at the 3' end of the  
fragment; and

5 where the family of fragments include at least one fragment terminating at each  
possible base, represented by either of the two non-Watson-Crick-pairing dideoxyterminators  
of step j) of that portion of the selected region of both template strands flanked by a primer of  
step j);

10 k) determining the location and identity of at least some of the bases in the  
corresponding region of the second polynucleotide.

54. The method of claim 53, where the sequence of the corresponding region of  
the second polynucleotide is determined simultaneously with determining the location and  
identity of the bases in the selected region of the first polynucleotide.

15 55. A method of claim 53, where producing the family of labeled, linear  
polynucleotide fragments in step e) and producing the family of labeled, linear polynucleotide  
fragments in step j) is performed in one reaction.

56. The method of claim 53, where each of the primer pair in step j) is labeled,  
and the labels on each of the primer pair in step j) are all distinguishable from each other.

20 57. The method of claim 53, where at least a portion of one of the dATP,  
dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step j) is labeled.

58. The method of claim 53, where each of the two non-Watson-Crick-pairing  
dideoxyterminators in step j) is labeled, and where each of the labels on the two non-Watson-  
Crick-pairing dideoxyterminators in step j) are all distinguishable from each other.

25 59. The method of claim 53, where each of the primer pair in step j) is labeled,  
at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP  
and dUTP in step j) is labeled, and where the labels on each of the primer pair in step j) and

the labels on at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step j) are all distinguishable from each other.

60. The method of claim 53, where each of the primer pair in step j) is labeled, each of the two non-Watson-Crick-pairing dideoxyterminators in step j) is labeled, and where  
5 the labels on each of the primer pair in step j) and each of the labels on the two non-Watson-Crick-pairing dideoxyterminators in step j) are all distinguishable from each other.

61. The method of claim 53, where at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step j) is labeled, each of the two non-Watson-Crick-pairing dideoxyterminators in step j) is labeled, and where the  
10 labels on at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step j) and each of the labels on the two non-Watson-Crick-pairing dideoxyterminators in step j) are all distinguishable from each other.

62. The method of claim 53, where each of the primer pair in step d) is labeled, each of the primer pair in step j) is labeled, at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step d) is labeled, at least a portion of  
15 one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step j) is labeled, each of the two non-Watson-Crick-pairing dideoxyterminators in step d) is labeled, each of the two non-Watson-Crick-pairing dideoxyterminators in step j) is labeled, and where the labels on each of the primer pair in step d), the labels on each of the primer pair in step j),  
20 the labels on at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step d), the labels on at least a portion of one of the dATP, dCTP, dGTP and either dTTP or dUTP or both dTTP and dUTP in step j), each of the labels on the two non-Watson-Crick-pairing dideoxyterminators in step d), and each of the labels on the two non-Watson-Crick-pairing dideoxyterminators in step j) are all distinguishable from each other.